# Tumor Vasculature as a Therapeutic Target in Non-small Cell Lung Cancer

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**Introduction:** We aim to describe the molecular mechanisms relevant to angiogenesis inhibition and to critically evaluate the current evidence for the use of angiogenic inhibitors (AIs) in the treatment of non-small cell lung cancer (NSCLC).

**Methods:** The literature on the basic concepts of tumor angiogenesis is reviewed. Published articles and major lung cancer conference abstracts were screened for reports on the use of AI in NSCLC patients and the National Institutes of Health clinical trials database was searched for relevant ongoing studies.

**Results:** We delineate in this review the molecular and cellular aspects of angiogenesis and vasculogenesis and outline the relevance of these to lung cancer. Clinical studies of AIs in NSCLC reported to date as well ongoing studies are summarized. Major issues discussed include the choice of the right molecular target; characteristics of various tyrosine kinase inhibitors; potential drawbacks and concerns regarding the application of AIs in clinical practice, and major unanswered questions and future directions.

**Conclusions:** AIs have antitumor activity in NSCLC and have become part of the standard of care for patients with advanced nonsquamous cell carcinoma. Unfortunately, the gains have been modest and robust predictive biomarkers are urgently needed. Clinical trials to date have validated the tumor vasculature as a legitimate target, and as our understanding of the biology of tumor angiogenesis increases, exciting new therapeutic approaches are being explored.

**Key Words:** Angiogenic inhibitors, Non-small cell lung cancer, Vascular endothelial growth factor, Tyrosine kinase inhibitors, Predictive biomarkers.

(*J Thorac Oncol.* 2012;7: 609–620)

## Lung Cancer Angiogenesis

As a malignant growth exceeds the size of a few 100 $\mu$m, nutrient diffusion becomes a growth-limiting factor. Hypoxia and cancer-specific genetic abnormalities, mainly by up-regulation of the hypoxia inducible factors, drive the secretion of proangiogenic factors (e.g., vascular endothelial growth factor [VEGF], basic fibroblast growth factor [FGF]) and the suppression of antiangiogenic factors (e.g., thrombospondin, endostatin), making the tumor microenvironment proangiogenic. New blood vessels are formed, and existing blood vessels are modified to provide blood supply to the tumor. The sprouting of blood vessels from existing blood vessels is called angiogenesis, whereas production of de novo blood vessels is termed vasculogenesis. Both processes are controlled by the counteracting effects of proangiogenic and antiangiogenic factors. The tipping of the balance toward a proangiogenic state, "the angiogenic switch," is essential for cancer progression. Unlike in physiological angiogenesis, reperfusion and reoxygenation do not turn off cancer angiogenesis, which is driven by tumor-secreted factors. Tumoral angiogenesis was suggested as a therapeutic target 40 years ago.[1] However, only recently have angiogenesis inhibitors been added to the available therapeutic armamentarium against colon, breast, kidney, brain, and other cancers. Figure 1 depicts some of the major cellular and molecular factors in cancer angiogenesis.

Histological evidence of enhanced angiogenesis in lung tumors has been associated with a poor prognosis[2,3] and levels of various molecular mediators of the angiogenic switch correlate with poor clinical outcome.[4] The clinical importance of angiogenesis inhibition in the treatment of non-small cell lung cancer (NSCLC) has been demonstrated.[5] However, it should be noted that a subgroup of NSCLC with nonangiogenic pattern has also been described.[6–8] These tumors seem to co-opt existing blood and lymphatic vessels rather than induce angiogenesis, and importantly have a worse prognosis than their angiogenic counterparts. Hence, it is unlikely that antiangiogenic treatments would be effective for all lung cancer patients.

## Major Molecular Mediators of Angiogenesis

Angiogenesis involves multiple cellular events and many interactions among a variety of cell types. A large number of molecules have been identified as modulators of processes required for the enhancement of tumor perfusion. A selected number will be mentioned below. For a recent comprehensive review of molecular and cellular mediators, see Ref. 9.

### Vascular endothelial growth factor

Initially named vascular permeability factor, VEGF is a glycoprotein that induces endothelial permeability and func-

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Disclosures: Glenwood D. Goss received support from AstraZeneca, Roche, Bristol-Myers Squibb, Boehringer Ingelheim, Pfizer, and OSI Pharmaceuticals.

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ISSN: 1556-0864/12/0703-0609

tions as a mitogenic and survival factor of endothelial cells.[10] There are five family members in mammalian cells, VEGF-A to -D and placental growth factor (PlGF). VEGF-A is known also as VEGF, and we will use this term in this review. Five isoforms of VEGF exist ($VEGF_{121}$, $VEGF_{145}$, $VEGF_{165}$, $VEGF_{189}$, and $VEGF_{206}$), differing by their affinity to the extracellular matrix and in their clinical importance.[11]

VEGF binds Flt-1/VEGFR-1 and Flk-1/VEGFR-2/KDR (kinase domain region), tyrosine kinase receptors, expressed by vascular endothelial cells, and by tumor cells and some epithelial cells. VEGFR-2 is a dominant positive regulator of the vascular system. VEGFR-1 has a higher binding affinity for VEGF than VEGFR-2 but lower kinase activity and no mitogenic response. VEGFR-1 knock-out mice die of overgrowth of endothelial progenitors.[12] In contrast, mice expressing a kinase-dead-VEGFR-1 develop normally, suggesting that this protein is a required negative regulator of the VEGF pathway, acting by sequestering ligand molecules.[13] However, other models demonstrate VEGFR-1 to be a positive angiogenesis regulator,[14] important in the recruitment of circulating endothelial precursors[15] and of inflammatory cells, which in turn secrete proangiogenic mediators. In addition, VEGFR-1 and VEGFR-2 cross-phosphorylate and activate each other when activated by PlGF and VEGF, respectively. The precise role of VEGFR-1 might be context dependant.

Much less is known about the other members of the VEGF pathway. VEGF-C and VEGF-D mainly regulate lymph vessel formation through activation of VEGFR-3,[16] although VEGFR-3 activity also seems to be required for blood vessel angiogenesis.[17]

VEGFR-2 ligand binding causes receptor dimerization, tyrosine phosphorylation, and activation of downstream signaling. Neuropilin-1 is a modulating coreceptor of VEGFR-2 activity. High neuropilin-1 mRNA levels in NSCLC tumors were found to be an independent negative prognostic factor.[18] Another modulator of VEGFR is vascular endothelial-cadherin, which forms a complex with VEGFR-2, $\beta$-catenin, and PI3K and is required for the transmission of the survival signal.[19] The VEGF pathway is a major regulator of angiogenesis, cross-talking with other growth factor signaling pathways, some of which are mentioned below.

## Platelet-derived growth factor

The platelet-derived growth factor (PDGF) ligands, secreted by tumor cells and endothelial cells, activate PDGF receptors, expressed by pericytes, thus recruiting them to developing vessels. Pericytes have a supportive and modulating role for blood vessels. Abnormal pericyte vessel coverage in PDGF-B null mice results in vessel dilatation, leakage, and hemorrhage.[20] The PDGF pathway is also involved in cancer-associated fibroblasts signaling and in autocrine cancer stimulation.[9] The importance of the PDGF pathway in NSCLC is supported by the correlation found between high tumor PDGF expression levels and poor prognosis.[21] Preclinical studies indicated enhanced antiangiogenic efficacy of a combined inhibition of VEGFR and PDGFR$\beta$.[22] Notably, in some cases, platelet-derived endothelial cell growth factor, which is the enzyme thymidine phosphorylase, produced by the *TYMP* gene, is mistakenly referred to as PDGF.

## Angiopoietin (Ang)

The angiopoietin family is composed of four ligands that bind the Tie-2 tyrosine kinase receptor. Ang-1 and Ang-4 function mostly as positive regulators, whereas Ang-2 and Ang-3 are mostly antiangiogenic. However, these roles are context dependent. Ang-2 antagonizes the Ang-1-dependent recruitment of pericytes to new blood vessels, thus preventing their stabilization. Ang-2 knockout mice have defects in adult vascular sprouting, suggesting that destabilization of the vessel structure is required for angiogenesis to progress. Ang-2 seems to have a proangiogenic role when VEGF is abundant but an antiangiogenic role when VEGF levels are low.[23] High tumor Ang-2 expression has negative prognostic implications in lung cancer patients, especially when VEGF expression is high.[24] Ang-1 has a negative role in tumor angiogenesis, probably secondary to enhanced pericyte vessel coverage and reduced vessel permeability. The resultant vessels do not allow extravasation of plasma proteins and the microenvironment formed is less proangiogenic.

## Endogenous antiangiogenic factors

Potent antiangiogenic factors can be produced endogenously by cleavage of other proteins. For example, angiostatin, a potent angiogenesis inhibitor, is the cleavage product of plasminogen, a component of the coagulation control mechanism. Macrophage-derived methalloelastase (MMP-12) is thought to be responsible for the in vivo conversion of plasminogen to angiostatin.[25] Thus, tumor-infiltrating macrophages may determine the production of antiangiogenic factors. In addition, cancer cells may secrete enzymes required for the production of angiostatin. Endostatin is an antiangiogenic factor that is a fragment of collagen-XVIII. Additional potent antiangiogenic factors are produced by the cleavage of common proteins,[26] suggesting that the tight control of angiogenesis requires reserves of antiangiogenic factors ready for rapid mobilization. Thrombospondin-1 is another endogenous angiogenic inhibitor (AI), mimetics of which are in early clinical trials.[27]

## Cell-cell adhesion molecules

The formation of new blood vessels requires interactions among endothelial cells, pericytes, smooth muscle cells, inflammatory cells, and epithelial cells and involves cell-cell adhesion molecules. Intercellular adhesion molecule 1 (ICAM-1) is a transmembrane protein involved in endothelial cell survival and migration. Plasma ICAM levels were found to be prognostic in a study of lung cancer patients treated with an anti-VEGF antibody.[28] N-Cadherin is another cell–cell interaction molecule being evaluated currently as a target for antiangiogenic treatment.[29]

## Growth factors and cytokines

Basic FGF is a potent angiogenic factor that stimulates the proliferation and migration of endothelial cells and the production of matrix metalloproteases (MMPs). Importantly, resistance to VEGFR inhibition can arise from up-regulation of FGF signaling.[30] Cytokines also modulate angiogenesis, conceivably by regulating leukocyte recruitment. Interleukin

(IL)-8, IL-12,[31] and transforming growth factor $\beta$ are examples of cytokines found to correlate with lung cancer angiogenesis. These and other cytokines are being investigated as predictive factors of efficacy or as therapeutic targets.[32,33]

## Agents Targeting Angiogenesis in Lung Cancer

### Targeting the VEGF ligand

Bevacizumab ("Avastin," Roche, Basel, Switzerland) is a monoclonal antibody directed against the VEGF ligand (VEGF-A specific). It was the first bonafide AI to be approved for cancer treatment, initially in colorectal carcinoma. Single agent bevacuzumab improved progression-free survival (PFS) in a few trials but seemed inefficient by itself in most cases, and its administration to NSCLC patients in combination with chemotherapy was tested. A phase II study comparing carboplatin and paclitaxel with or without bevacizumab demonstrated improved PFS and a trend of improved overall survival (OS) in the experimental arm[34] and led to a phase III trial. Because of cases of fatal pulmonary hemorrhages in squamous cell carcinoma patients in the phase II trial, bevacizumab was henceforth mostly tested in nonsquamous cell lung cancers and is currently approved only for nonsquamous cell lung cancers.

The Eastern Cooperative Oncology Group evaluated bevacizumab in a phase III study of 878 advanced nonsquamous-cell cancer patients (ECOG 4599) randomized to receive paclitaxel and carboplatin with or without bevacizumab. There was a statistically significant increase in median survival from 10.3 to 12.3 months favoring the bevacizumab arm.[5] This trial lead to the approval of bevacizumab for advanced nonsquamous NSCLC as first line regimen. Bevacizumab treatment was also evaluated in combination with cisplatin and gemcitabine (the AVAiL study) in 1050 advanced NSCLC patients and was shown to significantly increase PFS from 6.1 to 6.5 or 6.7 months (in the high and low dose groups, respectively), thus meeting its primary end point. No difference in OS was found in this study.[35] The reason for the difference in the results of the AVAiL and the E4599 is not known but could be due to differences in the patient cohorts, the inferior activity of paclitaxel and carboplatin versus cisplatin and gemcitabine, and the in vitro data supporting synergy between taxanes and bevacizumab.[36] In the BeTa-lung phase III trial, bevacizumab was added to erlotinib ("Tarceva," Roche, Basel, Switzerland) as second-line treatment. PFS was improved from 1.7 to 3.4 months, but only a trend toward survival benefit, the trial's primary end point, was shown.[37] Recently, two studies of bevacizumab combined with chemoradiotherapy have reported an alarmingly high rate of tracheoesophageal fistula formation.[38] However, another study has reported acceptable toxicity[39] and studies are ongoing. As with many other agents that demonstrate activity in the advanced setting, bevacizumab is also being evaluated in the adjuvant setting, in the randomized phase III study ECOG 1505 (NCT00324805). Phase III studies testing AIs are summarized in Table 1 and selected phase I and II studies are presented in Table 2.

VEGF-trap ("Aflibercept," NSC-724770, AVE0005, Regeneron, Inc., Sanofi-Aventis, Paris, France) is a novel agent designed to function similarly to bevacizumab. VEGF-trap is a recombinant protein, similar to the extracellular-ligand binding domains of VEGFR-1 and 2. It binds all VEGF isoforms and binds VEGF and VEGF-B with a higher affinity than bevacizumab. A phase II study reported some responses in heavily pretreated patients,[61] leading to an ongoing phase III trial, testing the addition of aflibercept to docetaxel as a second-line treatment (NCT00532155).

### Targeting the VEGF receptors

Fueled by the success of bevacizumab in advanced NSCLC and by evidence of efficacy of VEGFR inhibitors in other malignancies, much effort have been invested in testing VEGFR inhibitors in NSCLC. Most appealing is the possibility of inhibiting several of the pathways involved in angiognesis simultaneously. This can be achieved by tyrosine kinase inhibitors (TKIs), which frequently target multiple tyrosine kinase receptors. Most of them co-target the VEGFRs, PDGFRs, and c-Kit, related to their common split-kinase structure. TKIs have the convenience of oral administration. Their short half-life necessitates daily dosing but facilitates the control of reversible toxicities. Bioavailablilty, pharmacokinetic, and pharmacodynamic properties contribute to differences among the TKIs. Importantly, multikinase inhibition might be antagonistic in some cases. For example, if inhibiting VEGFR-1 would have in tumors a similar effect as knocking it out in mice, enhanced angiogenesis might be expected. Most TKIs, including some claiming high specificity, actually inhibit a significant number of kinases, as demonstrated in an in vitro study testing the affect of 38 TKIs on a set of 317 kinases.[62] Because of the technical challenges inherent in the assessment of kinase inhibition, indirect comparisons of different TKIs, and TKI-defined target claims, should be evaluated cautiously. This group of agents includes most of AI agents tested in clinical trials today (Table 1). Below, we discuss aspects of some of these TKIs.

Sunitinib (SU11248, Pfizer, New York, NY) was developed as an oral inhibitor of VEGFR-1–3, PDGFR-$\alpha$ and $\beta$, and c-Kit receptor. More extensive molecular analysis demonstrates it to be one of the most promiscuous TKIs, inhibiting 18% of the 317 tested kinases.[62] An approved drug for the treatment of gastrointestinal stromal tumor and metastatic renal cell carcinoma (RCCa), it is being tested now as a treatment for NSCLC. Sorafenib (Nexavar, Bayer, Germany) is another TKI, also approved as a treatment for RCCa and for hepatocellular carcinoma. It was designed to target Raf kinase but found to inhibit also VEGFR-1–3, PDGFR, RET, KIT, and FLT-3. A phase III trial comparing chemotherapy with and without sorafenib (ESCAPE trial), closed after it failed to show any benefit with the sorafenib combination.[63] Importantly, in the squamous-cell cancer subgroup of patients ($N = 219$), sorafenib-treated patients' survival was reduced from 13.7 to 8.9 months. This is reminiscent of the squamous-cell specific detrimental effect seen with bevacizumab and also noted with Motesanib (AMG706, Amgen, CA) another VEGFR/PDGFR TKI. A detrimental effect of some AI drugs in a subset of patients does not indicate necessarily that this AI or the whole class should be abandoned; rather, it stress the need for biomarkers that will target those drugs to

 

**TABLE 1.** Phase III Studies Evaluating Antiangiogenic Treatments

| Drug | Tx Line | Treatment | Experimental Arms | Remarks | Name of Study/REF/Clinical Trial, NIH Website |
|---|---|---|---|---|---|
| Bevacizumab | I | Carboplatin-paclitaxel | Bevacizumab/placebo | Improved OS (10.3–12.3 mo) | ECOG 4599[5] |
| Bevacizumab | I | Cisplatin-gemcitabine | Bevacizumab/placebo | No OS improvement | AVaIL[35] |
| Bevacizumab | II | Erlotinib | Bevacizumab/placebo | Second line Tx. A trend of OS improvement | BeTa-Lung[37] |
| Bevacizumab | Adj | Adj chemotherapy | Bevacizumab/placebo | Various platinum combinations allowed, recruiting | NCT00324805 |
| Ramucirumab | II | Docetaxel | Ramucirumab/placebo | Recruiting | NCT01168973 |
| Aflibercept | II | Docetaxel | Aflibercept/placebo | Ongoing, not recruiting | VITAL, NCT00532155 |
| Sunitinib | II-III | Erlotinib | Sunitinib/placebo | Second or third line treatment, no OS benefit | NCT00457392[40] |
| Sunitinib | Maint | Maint after cisplatin-based chemotherapy | Sunitinib/placebo | Recruiting | NCT00693992 |
| Sorafenib | I | Carboplatin-paclitaxel | Sorafenib/placebo | Stopped at interim analysis: failed, higher mortality in SCCa patients | ESCAPE, NCT00300885 |
| Sorafenib | I | Cisplatin-gemcitabine | Sorafenib/placebo | Squamous cell carcinoma pts withdrawn. No OS or PFS benefit. | NExUS, NCT00449033[41] |
| Cediranib | I | Carboplatin-paclitaxel | Cediranib 30 mg/placebo | Improved RR, higher treatment related mortality | BR.24[42] |
| Cediranib | I | Carboplatin-paclitaxel | Cediranib 20 mg/placebo | Recruiting | BR.29, NCT00795340 |
| Motesanib (AMG-706) | I | Carboplatin-paclitaxel | Motesanib/placebo | Recruiting. SCCa patients excluded after interim safety review. | MONET1, NCT00460317 |
| Pazopanib | Adj | | Pazopanib/placebo | Recruiting. stage I, $T \leq 5$ cm, N0. 24 wk treatment | NCT00775307 |
| Pazopanib | Maint | | Pazopanib/placebo | Nonprogressors after first line Tx. Treatment till progression or toxicity | NCT01208064 |
| Vandetanib | II | Docetaxel | Vandetanib/placebo | Increase PFS but not OS, endpoint of study met | Zodiac[43,44] |
| Vandetanib | II | Pemetrexed | Vandetanib/placebo | No improvement | ZEAL[45] |
| Vandetanib | II-III | Erlotinib | Vandetanib/Erlotinib | Noninferior | ZEST[46] |
| Vandetanib | II-III | Best supportive care | ±Vandetanib | EGFR TKI failure patients, OS similar to placebo | Zephyr[47] |
| BIBF-1120 (Vargatef) | II | Second line treatment with docetaxel | BIBF-1120/placebo | Recruiting | LUME-lung 1, NCT00805194 |
| BIBF-1120 (Vargatef) | II | Second line treatment with pemetrexed | BIBF-1120/placebo | Recruiting | LUME-lung 2, NCT00806819 |
| Endostar | I | Vinorelbine-cisplatin | Recombinant endostatin/placebo | Improved RR and TTP | Ref. 48 |
| Endostar | Adj | Cisplatin and vinorelbine | ±Endostar | Recruiting, stages Ib-IIIa. | NCT00576914[49] |
| Endostar | I | Cisplatin and docetaxel | ±Endostar | Recruiting | NCT00657423 |
| ASA404 | I | Carboplatin-paclitaxel | ASA404/placebo | Terminated after a negative interim analysis | ATTRACT1, NCT00662597[50] |
| ASA404 | II | Docetaxel | ASA404/placebo | Terminated after a negative interim analysis | ATTRACT2, NCT00738387[51] |
| Thalidomide | I | Gemcitabine-carboplatin | Thalidomide/placebo (2 yr) | No benefit. Reduced survival in nonsquamous cancer patients | Ref. 52 |

Tx, treatment; OS, overall survival; PFS, progression free survival; RR, response rate; EGFR, endothelial growth factor receptor; TKI, tyrosine kinase inhibitor; Maint, maintenance; Adj, adjuvant; TTP, time to progression.

**TABLE 2.** Selected Phase I and II Studies Using AIs

| Molecular Mechanism | Name of Drug | Company | Phase I | Phase II | Randomized Phase II |
|---|---|---|---|---|---|
| Peptibody: ang inhibitor | CovX | Pfizer, NY | Ongoing[53] | | |
| Peptibody: ang inhibitor | AMG 386 | Amgen, CA | Ongoing[54] | | |
| Thrombospondin1 mimic | Cvx-045 | Pfizer, NY | Ongoing, not recruiting[27] | | |
| Inhibition of VEGF expression | PTC299 | PTC therapeutics, NJ | Ongoing[55] | | |
| Antibody against ALK1 | PF-03446962 | Pfizer, NY | Ongoing[56] | | |
| Cyclic peptide: N-Cadherin inhibitor | ADH-1, Exherin | Adherex, Durham, NC | Ongoing, not recruiting NCT00265057 | | |
| TKI: VEGFR-1–3, PDGFR-$\alpha$ and $\beta$ | Tivozanib (AV 951) | Aveo pharmaceuticals | Ongoing, not recruiting (phase I-II) NCT00826878 | | |
| Antibody against VEGF | Bevacizumab | Roche, Basel, Switzerland | With chemo-rads (phase I-II) NCT00334815 NCT00280150 NCT00369551 | With metronomic chemo NCT00655850 NCT00755170 NCT00755157 | |
| Antibody against VEGF | Bevacizumab | Roche, Basel, Switzerland | | With pemetrexed as second line for nonsquamous[57] | |
| TKI: VEGFR1–3, PDGFR, and c-Kit receptor | Pazopanib (GW786034) | Glaxo-SmithKline, UK | | Single agent third line NCT01049776 | Combinations first line, NCT00866528 second line NCT01027598 NCT00871403 NCT01107652 |
| Antibody against VEGFR-2 | Ramucirumab | Eli Lilly, IN | | With chemo[58] | With chemo NCT01160744 |
| Antibody against phosphatidylserine | Bavituximab | Peregrine, CA | | | With chemo NCT01160601 NCT01138163 |
| TKI: FGFR and EGFR | Brivanib (BMS-582664) | Bristol-Myers Squibb, NY | | | Randomized withdrawal NCT00633789 |
| TKI: VEGFR1–3, PDGFR, c-Kit receptor, and FLT3 | Linifanib (ABT-869) | Abbott, IL | | | First line with chemo[59] NCT00716534 |
| TKI: VEGFR1–3, PDGFR, and c-Kit receptor | Axitinib (AG013736) | Pfizer, NY | | | First line, with chemo NCT00768755 NCT00600821 |
| Vascular disrupting agent | Fosbretabulin, (CA4P) | OXiGENE, CA | | | With chemo and bevacizumab NCT00653939 |

Peptibody—a hybrid molecule of a targeted peptide and a stabilizing Fc component of an antibody.[60]
TKI, tyrosine kinase inhibitor; VEGFR, vascular endothelial growth factor receptor; Carbo, carboplatin; Chemo-rads, chemotherapy-radiotherapy; Chemo, chemotherapy.

the patients most likely to benefit from it and prevent its use in patients that would be harmed by it. Cediranib (AZD2171, "RECENTIN," AstraZeneca, London, UK), another oral inhibitor of VEGFR-1–3, PDGFR-$\beta$, and c-Kit receptor, is an example of this point. A phase II–III trial (BR.24, NCIC-CTG) examined the addition of this agent to a carboplatin and paclitaxel regimen.[42] Significant toxicities required a reduction of the cediranib dose (from 45 to 30 mg daily). Furthermore, although the death rate was similar in the two arms of the study, a higher rate of treatment-related death was noted in the cediranib arm. However, the response rate increased from 16 to 38% and the hazard ratio for progression-free survival was 0.77 in favor of the cediranib arm[42] (in the 30 mg cohort, the median progression-free survival was 5.6 months versus 5 months; in the 45 mg cohort, 6.05 versus 5.45 months in the cediranib and placebo arms, respectively). A positive impact of cediranib was seen regardless of gender, histology, or smoking status. Baseline weight loss of more than 5% and hypoalbuminemia predicted increased toxicity for patients receiving cediranib. This led to the initiation of a second trial similar in design, using a lower dose (20 mg) of cediranib, and excluding patients with a weight loss of more than 5% (NCIC-CTG BR.29). Using this approach, the investigators hope to sustain the positive impact of cediranib while reducing toxicity.

An example of a non-TKI that targets the VEGFR is Ramucirumab (Eli Lilly, IN), a novel antibody directed against VEGFR-2, unlike bevacizumab that targets the VEGF ligand.[58] Directed against the extracellular component of the receptor, its mechanism of inhibition is different than the TKIs, thus in theory both might be applied in concert.

## Targeting the EGFR and additional receptors

Vandetanib (ZD6474, "Zactima," AstraZeneca) is an oral inhibitor of VEGFR-2, 3, Ret kinase, and EGFR, conceived with the idea that blocking both angiogenesis and tumor cell proliferation would be synergistic. This drug showed promising activity in advanced NSCLC in several phase II trials, leading to four recently reported phase III studies. None of these trials demonstrated an improvement in survival for patients receiving vandetanib, although the ZODIAC trial met its end point of improved PFS (Table 1). The failure of vandetanib to improve OS might be related to the fact that it inhibits EGFR about 10-fold less than it does VEGFR2[64] and thus might not actually target both intended pathways. XL 647 (Exelixis, CA) is another oral TKI that targets the VEGFR and EGFR pathways, and inhibits Her2 and EphB4, that was tested in NSCLC patients, demonstrating moderate activity in patients harboring EGFR mutations.[65] Brivanib (BMS-582664, Brisol-Myers Squibb, NY) is a TKI targeting FGFR and EGFR, thus targeting both angiogenesis and tumor cell proliferation, currently in phase II trials (NCT00633789).

Combining different agents is another manner of inhibiting both the EGFR and the VEGFR pathways. Sorafenib addition to erlotinib treatment did not improve disease-free survival although disease control rate was better (D. Spigel, personal communication, February 2010). Cetuximab with bevacizumab and chemotherapy[66] is currently being evaluated (NCT00946712), bevacizumab being given to all eligible patients, and randomization done between addition of cetuximab or not. It should be noted that addition of cetuximab to a bevacizumab-chemotherapy regimen worsened outcomes of colorectal cancer patients in two recent trials.[67,68]

## Targeting existing blood vessels (vascular disrupting agents)

Unlike AIs that aim to prevent the sprouting of vessels or the production of new ones, vascular disrupting agents (VDA) target existing blood vessels. VDA can bring about a collapse of the tumor's vascular supply and massive necrosis within hours. Besides the title, various VDA have little in common, embracing a range of mechanisms of action. There are antibodies or peptides that target toxins to tumor-vasculature-specific epitopes, tubulin-binding molecules that target dividing cells and others that are activators of cytokine production. A characteristic observation in studies of VDA is the viable rim of live tumor cells, the culprit of repopulation that remains around an area of central necrosis.[69] For this reason, VDAs are considered best combined with chemotherapy, radiotherapy, AIs, or other agents that would disrupt this viable rim. Combretastatin A4 phosphate (CA4P, "fosbretabulin," OXiGENE, CA) inhibits microtubule assembly, whereas 5,6-dimethylxantlenone-4-acetic acid (DMXAA, ASA404, vadimezan) functions through a different mechanism, partly by activating interferon-$\beta$. Originally isolated from the South African willow tree Combretum caffrum, these agents block tumor blood flow rapidly by causing vasoconstriction of tumor-feeding arterioles, or by endothelial cell apoptosis.[70] A promising randomized phase II study with ASA404 has lead to two phase III trials, both of them recently reported as negative.[50,51] CA4P is currently in phase II studies.[71] ABT-751 is a novel antimicrotubule agent also defined as VDA. A phase I–II of this agent in combination with pemetrexed was recently reported with interesting evidence of activity in squamous cell carcinoma patients.[72]

## Targeting angiogenesis using chemotherapy

In in vivo models, chemotherapy given at doses much lower than the maximal tolerated dose, but on a continuous basis (metronomic treatment), has a marked antiangiogenic effect.[73] Ongoing trials are evaluating this strategy. Some chemotherapy agents targeting the cytoskeleton are potent endothelial cells toxins, e.g., paclitaxel and docetaxel.[74,75] Furthermore, paclitaxel and docetaxel cause a reduction of interstitial fluid pressure (IFP) by killing tumor cells, thus allowing better tissue perfusion and better drug delivery.[76]

## Targeting other angiogenic mechanisms

A recombinant version of endostatin, an endogenous antiangiogenic factor discussed above, was evaluated in 42 patients with neuroendocrine tumors with no documented responses.[77] Endostar, (YH-16, Simcere, China) is a recombinant human endostatin modified by the addition of nine amino acids, thus simplifying the purification process and improving stability. In a study of 493 advanced NSCLC patients, addition of endostatin to chemotherapy led to increased time to progression, from 3.6 to 6.3 months.[48] Recently approved in China for NSCLC patients, it is being

tested in various settings including two phase III trials in metastatic disease.

N-Cadherin is a cell-cell adhesion molecule, expressed by endothelial cells and some cancer cells. ADH-1 is an inhibitor of N-Cadherin (Exherin, Adherex, Durham, NC) that was recently tested in phase I–II studies that included NSCLC patients (NCT00265057, NCT00264433). Thalidomide has an antiangiogenic activity that is poorly understood and may act indirectly through its immunomodulatory effects. A negative phase III study testing its role in NSCLC was recently published.[52]

Targeting tumor vasculature-specific proteins is an additional approach which seems appealing as a therapeutic strategy. Tumor-blood-vessel-specific proteins and phospholipids have been identified. One of these is phosphatidylserine, normally found only in the inner lipid leaflet of the cell membrane but translocates to the outer leaflet in tumor vasculature. It is currently targeted in the clinic by a novel specific antibody (bavituximab, Peregrine, CA).[78]

## Insight from Studies Using AIs in Lung Cancer

### Resistant angiogenesis

Although many AIs show promising responses and PFS improvements in NSCLC patients, almost none bring about prolongation of survival. The reasons for this phenomenon are not known. A relevant observation might be rebound angiogenesis, observed in mice studies where the VEGF pathway was effectively attenuated.[30] In these models, alternative angiogenic mechanisms are up-regulated, including induction of FGF family members, angiopoietins, and vessel co-option, possibly triggered by hypoxia in tumors subjected to VEGF inhibition.[79] Indeed, up-regulation of one of several alternative angiogenic pathways has been demonstrated in human lung cancers.[80] Another important finding in models of VEGF-dependent tumor growth treated with VEGF inhibitors is increased invasiveness of tumors that thrive in these conditions.[30,81] This could be secondary to hypoxia-induced activation of the hepatocyte growth factor-Met pathway,[82] urokinase-type plasminogen activator,[83] or other survival pathways. Furthermore, hypoxia may select for tumor cells with an aggressiveness phenotype, e.g., those with loss of p53[84] or other genetic events.[85] Two recent studies in mice models indicate a risk of enhanced metastatic spread as a result of AI treatment.[86,87] Using VEGF pathway inhibition as a cancer therapeutic strategy, requires further understanding about cancer escape and resistance mechanisms.

### The requirement for a continuous treatment

The importance of treatment schedules of AIs is currently not clear. Rebound accelerated tumor growth is seen on discontinuation of bevacizumab treatment of metastatic colorectal cancer[88] and in patients with glioblastoma multiforme during drug holiday of a VEGFR inhibitor.[89] Sunitinib and axitinib treatment breaks result in increase tumor perfusion and proliferation.[90] In a retrospective comparison of two cohorts of a sunitinib phase II study, more responses were seen in a noncontinuous, higher dose treatment, but longer survival was observed in the cohort treated continuously,[91] supporting the principle of daily administration of AI drugs. Regarding the length of treatment, current practice with most AIs is continuous treatment till progression. Supportive of this, maintenance bevacizumab prolonged PFS in a recent phase III trial of ovarian, peritoneal, and fallopian carcinomas.[92] When combined with chemotherapy, it is not known whether AIs should be continued beyond progression. We are not aware of any studies designed to answer this question.

### Combining AI treatments with chemotherapy

The only trial where an AI prolonged the life of NSCLC patients involved administering bevacizumab with chemotherapy. However, when an AI agent is given concurrently with a cytotoxic agent, antagonism is expected, because damaging the tumor's blood supply should hinder delivery of the cytotoxic agents. A possible explanation for the apparently unexpected synergism observed clinically was suggested by Jain and others regarding the IFP of tumors.[93] The increased permeability typical of tumor vessels results in extravasation of macromolecules and fluid to the extravascular compartment and increased IFP. This increased IFP reduces vascular flow and drug delivery. AIs cause a rapid normalization of tumor vasculature, reducing their permeability and the IFP. Thus, before elimination of the vessels that perfuse a tumor, a window of opportunity might exist when tumor perfusion and drug delivery is increased.[94] IFP is reduced after treatment with anti-VEGF antibody,[93] with a VDA,[95] and with sunitinib,[96] cediranib[89] and pazopanib (Votrient, GlaxoSmithKlin, UK).[97] However, improved tumor perfusion after such treatments remains a theoretical consideration and to date has not been consistently demonstrated by perfusion studies in humans. In contrast, axitinib decreased tumor exposure to concomitantly given cyclophosphamide,[98] and sunitinib treatment only enhanced day 3 diffusion parameters and not tumor perfusion.[99] On the basis of those observations, it can be speculated that an AI-drug holiday before chemotherapy administration might enhance chemotherapy delivery and treatment efficacy. However, AI and chemotherapy synergism might be secondary to enhanced killing of tumor endothelial cells,[4,75,98] suggesting they should be given concurrently. The requirement for an AI-drug holiday is being investigated in a phase I-II trial combining axitinib with cisplatin/pemetrexed (NCT00768755).

Taxanes were shown to mobilize bone marrow-derived circulating endothelial progenitor cells (CEPs) that colonize tumors and allow for regeneration of tumor vasculature after treatment.[36] Blocking the VEGF pathway prevents the CEPs surge and might be basis for chemotherapy-AI synergism, possibly limited to drugs that mobilize CEPs.

### Evaluating response to AI treatments

An apparent class effect of AI treatments is tumor cavitation, seen in 24% of treated tumors in one published series.[100] It seems plausible that assessing response solely according to RECIST criteria might misclassify cavitating tumors. An alternative single dimension measurement taking cavitation size into account was recently proposed.[100] Software-assisted volumetric measurement is an alternative approach. The role of these novel response measurements needs to be examined in large trials, comparing them to standard RECIST criteria.

## Toxicities of AIs

Less than 1% of adult endothelial cells are actively proliferating, suggesting that targeting endothelial proliferation would impact only tumor-activated endothelial cells and wound healing and be devoid of significant side effects. However, the toxicities seen with VEGFR inhibitors indicate an unappreciated role of VEGF in microvessels and tissue homeostasis. Further discussion of this topic is beyond the scope of this review. Interestingly, some toxicities might be useful as predictive markers of response to treatment.[101]

## Predicting Response to AI Treatments

In light of the potentially significant toxicities and lack of survival benefit in many of the AI trials, there is an urgent need for predictive biomarkers. In an analysis of the E4599 trial, VEGF plasma levels were predictive of response to bevacizumab but not predictive of a survival benefit.[28] In contrast, VEGF plasma levels were negatively correlated with PFS in patients treated with vandetanib,[102] and a greater increase in VEGF plasma levels with vandetanib treatment predicted worse outcome.[32] PlGF elevation showed a trend to be predictive of response to motesanib.[103] Polymorphisms in the *VEGF* gene, and in the *ICAM-1* and *WNK1* genes were found to correlate with bevacizumab-related improved survival.[104] An increase in ICAM-1 levels with treatment was associated with a better PFS in vandetanib-treated NSCLC patients.[32] Analysis of E4599 samples revealed improved PFS with bevacizumab for patients with low baseline levels of ICAM-1 and improved OS with bevacizumab for patients with stable levels of E-selectin.[28] Baseline levels of hepatocyte growth factor and of IL-12 were predictive of response to pazopanib.[33] Tumor mRNA levels of LDH-A, Glut-1, and VEGFR-1 were found to predictive of response to the VEGFR inhibitor PTK787/ZK 222584 in colorectal cancer patients.[105]

In vivo measurements of patients' tumor blood perfusion changes with initial doses of the antiangiogenic agent are potential predictive markers. Magnetic resonance imaging dynamic contrast enhancement measurements after 2 days of an oral antiangiogenic treatment were found to correlate with patients' drug exposure.[106] As early as 24 hours after the first oral dose of an angiogenesis inhibitor, a significant reduction of permeability and vessel size can be demonstrated.[89] The predictive power of these imaging studies is yet to be validated.

Clinical characteristics might also be predictive. Female patients did not benefit from bevacizumab in the E4599,[107] whereas an opposite trend was seen in the AVaiL trial[35] and in a phase II vandetanib trial[32] (a result not reproduced in the phase III vandetanib trial[43]). Adenocarcinoma patients had a clear benefit from bevacizumab, a result that could not be demonstrated for other nonsquamous histologies.[108] Recently, antiangiogenesis-induced arterial hypertension was found to correlate with clinical response and outcome in various tumors.[109–111] Retrospective analysis of the E4599 NSCLC trial demonstrates that HTN induced by bevacizumab correlates with a significantly improved OS[101] but conflicting results have been reported in colorectal cancer.[112] So far, none of the above mentioned candidate predictive biomarkers have been validated prospectively.

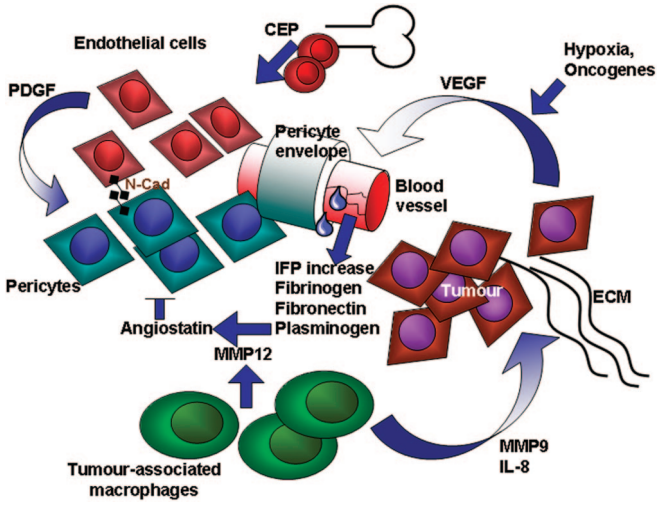


**FIGURE 1.** Vascular endothelial growth factor (VEGF) secretion by tumor cells is induced by tumor hypoxia and oncogene signaling.[4] VEGF acts mainly on endothelial cells, inducing angiogenesis and a hyperpermeable state. Tumor endothelial cells are one of the sources of platelet-derived growth factor, which recruit pericytes to the vessels' envelope. N-Cadherin (N-Cad) physically connects endothelial cells and pericytes.[29] Plasma proteins such as fibrinogen and fibronectin leak through the abnormal tumor vessels, contributing to the proangiogenic microenvironment and to the enhanced interstitial fluid pressure (IFP) typical of tumors.[94] Tumor-associated macrophages are a subtype of macrophages that invade cancer stroma and secrete MMP9, leading to release of extracellular matrix-associated VEGF[113]; these cells are also capable of promoting the formation of antiangiogenic factors such as angiostatin.[25] Circulating endothelial progenitors (CEP) are putative bone marrow-derived cells that home to tumor sites and might be a major contributor to the newly formed blood vessels.[69] This scheme represents a partial view of the major players involved in cancer angiogenesis.

## Summary and Future Directions

Angiogenesis inhibitors have obvious antitumor activity in NSCLC and bevacizumab has become part of the standard of care for patients with advanced nonsquamous cell carcinoma. Unfortunately, to date, the gains have been modest. As we learn more about angiogenesis, the complexity of the biology becomes apparent (Figure 1) and the number of unanswered questions increase (Table 3). As seen in many areas of medicine in the past, some of the answers may be surprisingly simple. New and exciting areas of research include elucidating the role of CEPs, tumor-vasculature specific molecules that allow their specific targeting, novel signaling pathways involved in angiogenesis and in vivo real-life imaging of the vasculature and tumor cell proliferation. These developments and others are eagerly awaited. In conclusion, the tumor vasculature remains an important area of anticancer research. Better understanding of the biology

**TABLE 3.** Nonresolved Issues Regarding AI for the Treatment of Lung Cancer

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| Nonresolved issues regarding combination treatments |
| Blocking multiple angiogenic factors (e.g., VEGFRs and PDGFRs) vs. blocking specific molecular mediators proven to be positive angiogenic regulators (e.g., blocking VEGFR-2 and not VEGFR-1)? |
| Blocking simultaneously the VEGF signaling pathway (targeting endothelial cells, achieving microvasculature normalization) and the PDGF pathway (targeting pericytes, preventing vessel maturation)? |
| Blocking simultaneously the extracellular component and internal domain of a relevant signaling pathway (e.g., bevacizumab and VEGFR TKI) to achieve complete blockage? |
| Blocking angiogenesis and proliferation pathways simultaneously (e.g., vandetanib), thus targeting both tumor cells and their blood supply? |
| Nonresolved issues regarding the preferred type of therapeutic molecule |
| Prefer small molecule TKIs |
| To allow multikinase targeting? |
| To allow rapid reversal of toxicities (short half-life)? |
| Prefer humanized antibodies/peptibodies |
| To allow specific single molecule targeting? |
| To enhance preferential tumor delivery (through enhanced permeability and retention effect[114])? |
| Nonresolved issues about combining AI with chemotherapy |
| Combine AI with chemotherapy |
| As a way to prevent CEP-mediated tumor salvage? |
| As a way to reduce IFP and improve chemotherapy delivery? |
| As a way to kill endothelial cells more efficiently? |
| The need for an AI drug holiday proceeding chemotherapy administration? |
| Continue AI agents after AI-chemo combined treatment, until disease progression? |
| Continue AI treatment beyond progression on AI-chemo combined treatment? |

AI, angiogensis inhibitor; chemo, chemotherapy; TKI, tyrosine kinase inhibitor; VEGF, vascular endothelial growth factor; IFP, interstitial fluid pressure; CEP, circulating endothelial progenitors.

and trials with angiogenesis inhibitors has already yielded positive results for NSCLC patients. Ongoing study of this exciting target is imperative.

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